Environmental Health Perspectives
Vol. 33, pp. 25-35, 1979

Intestinal Metabolism

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The anatomy of the intestine is outlined. The kinetics of cell proliferation, differentiation, and migration in intestinal epithelium are described. The distribution of cell types between the proliferation and differentiation compartments of the epithelium is summarized.

Seven preparations of intestine in current use for metabolic studies are listed. An outline review is presented of recent results on glucose, amino acid and ketone body metabolism obtained with three of these preparations: the ligature-isolated loop of intestine *in vivo*; the vascularly and luminally perfused intestine *in vitro*; and suspensions of respiring intestinal epithelial cells *in vitro*.

Reasons are given for thinking that these experimental systems are among the most useful currently available for metabolic studies, perhaps especially in the investigation of the metabolic fate of potentially toxic materials to which animal species may be exposed.

Introduction

Special attention has been given in recent years to the precise topology of the intestinal vasculature, particularly the microcirculation close to the epithelium; to the architecture of the intestinal epithelial cells; to the kinetics of epithelial cell proliferation, migration and differentiation; to the role of certain of these cells in catalyzing the terminal stages of gastrointestinal digestion, in absorbing solutes from the intestinal lumen and from the vascular bed, and in the translocation of water and solutes from the lumen to the circulation. Finally, there has been a revival of interest in those metabolic events which are a concomitant equally of digestion/absorption/translocation and of cell proliferation/differentiation/migration in the intestine.

This renewed interest has arisen in part from the emergence of new techniques, in part from a wider appreciation of the need for collaboration among different disciplines, and in part from the realization that intestine is an organ of critical importance to whole-body metabolism of normal and toxic materials.

This article is not intended as an exhaustive review of recent advances in our understanding of intestinal metabolism. It concentrates rather on results obtained by two relatively new experimental systems: the perfused intact intestine and suspensions of isolated intestinal epithelial cells. Quotations from the literature are selected rather than complete; they are intended to illustrate particular points and to provide the reader with access to other publications.

Anatomical Structure of Intestine

Detailed accounts of the structure of small and large intestine, of the arterial supply, venous and lymphatic drainage, and of the innervation of the tissue, will be found in any standard textbook of human anatomy. Corresponding detailed descriptions for common experimental animals are rare. A detailed account is available for the rat (*1*). The present description is intended solely to provide the necessary background for this article.

Three main components of intestinal anatomy can be discerned. The luminal surface of the tissue is covered with a continuous unicellular layer of epithelial cells arranged in pallisade fashion, each cell being fused to its immediate neighbors by tight junctions near its luminal pole and, elsewhere along the lateral cell-surface membrane, by desmosomes (*2, 3*). The immediate subepithelial component is mucosal tissue. In normal small intestine this is convoluted to form villi which project into the lumen and crypts which lie deep within the mucosa; in large intestine, the epithelium lines both the luminal surface proper and a regular series of pits inserted into the mucosal tissue. Circular and longitudinal muscle layers complete the major structural features of in-

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testine. Small and large intestine receive their arterial blood supply from three branches of the aorta: the coeliac axis, the superior and inferior mesenteric arteries. A tributary of the hepatic portal vein, the superior mesenteric vein, drains both small and large intestine. The microvascular arrangement of arterial supply and venous drainage within the villi of different species of small intestine has been described (*4*). Neural control of shunting of the blood supply past the villi has been described (*5*). The separate phenomenon of differential plasma skimming in the villus microvasculature has also been detailed (*6*). Each villus of the small intestine is drained by a lymphatic ductule. The lymphatic drainage of the large intestine, with that of the small intestine, eventually empties into the thoracic duct. These vascular and lymphatic structures permit the establishment of two important intestinal perfusion techniques mentioned below.

Large and small intestine are innervated by sympathetic and parasympathetic nerves; the nerve supply penetrates the mucosal tissue of each villus of the small intestine. Humoral control of intestinal functions has been reviewed (*7*). Intestinal epithelium itself has endocrine and exocrine functions (*8-11*).

The intestine is richly endowed with lymphoid tissue (Peyer's patches) and cell components of the immune system are embedded in the mucosa of small intestinal villi.

Intestinal Epithelium: Morphology; Kinetics of Cell Proliferation, Migration, and Differentiation

Detailed descriptions of the various epithelial cells (especially of the large intestine) have been given (*12*), and the fine structure of several intestinal epithelial cells has been reviewed (*8-10*).

A small population of stem cells has been identified in the crypts of small intestine (*13, 14*); these may be the origin of a few endocrine and exocrine cells found in the crypts, as well as the origin of a much larger population of proliferating cells found in the crypts immediately above the stem cells. It is well established that DNA replication, and mitotic division of epithelial cells, are confined to the lower half of the crypts of normal small intestine (*15-17*). The progeny of these cell divisions migrate into the upper crypts where differentiation begins and then continues as cells emerge in ordered array out of the crypts, onto and along the villi (*16*); senescent cells desquamate from the tips of the villi. A notable morphological feature of the cell differentiation process is the appearance of a pronounced brush border at the luminal pole of the columnar absorptive (brush border) cells as they leave the crypts. A number of hydrolytic enzymes known to be associated with the brush border increase in activity as differentiation and cell migration proceed (*18, 19*). Other enzymes not associated with brush borders show more complicated patterns of change in activities during cell differentiation in small and large intestine (*20-23*). Allied to these changes in enzyme activity which are associated specifically with short term cell differentiation are longer term changes associated with postnatal development (*24*). Circadian variation in intestinal enzyme activities, and changes in enzyme activities associated with changes in diet or exposure to xenobiotics have also been reported (*25-30*).

Distribution of Cell Types and Cell Components

Crypt cells account for about 25% and villus cells for about 75% of the total epithelial cell numbers of small intestine and for about the same proportion of the total DNA, RNA and protein. The crypt cell population is dominated by proliferating cells and differentiating cells most of which are destined to appear on the villi; a few remain in the crypts as differentiated endocrine and exocrine cells. Of the cells which populate the villi, the majority are columnar absorptive (brush border) cells, but some are mucin secreting "goblet" cells; other minor populations of cells are also present (*12*).

Choice of Experimental System

The preceding description emphasizes the contribution of a range of disciplines to our present understanding of intestinal structure, of neural and humoral control of intestinal activities, and of epithelial cell turnover. It is intended also to emphasize the need for care in several matters. First, this author prefers the terms duodenal, jejunal, ileal, and colonic brush-border cells to describe the functional absorptive cells of the different regions of the intestine, rather than the general term "enterocyte" which could refer to any one of several cell types in small or large intestine. Secondly, some caution is necessary in making deductions about events in such a complex tissue *in vivo* from results obtained with preparations of intestine *in vitro*. Thirdly, one must remain aware of the possible effects of diet, development, differentiation and circadian rhythms on results obtained in any preparation of intestine. Fourthly, selection of an intestinal preparation for metabolic studies requires care. Table 1 lists several established preparations. Preparations 5, 6, and 7 have undoubtedly contributed most to our current understanding of specific metabolic events in intestine, including those special aspects of metabolism

Table 1. Preparations of intestine in current use for metabolic studies.

No.	Type
1	Ligature-isolated segments of intestine *in vivo*.
2	Vascularly and luminally perfused intestine *in vitro*.
3	Suspensions of brush border cells incubated *in vitro* (cells differentiating *in vivo*); Suspensions of lower crypt cells incubated *in vitro* (cells proliferating *in vivo*).
4	Suspensions of brush border membrane vesicles incubated *in vitro;* suspensions of basolateral membrane vesicles incubated *in vitro*.
5	Everted sacs of intestine incubated *in vitro*; sheets of whole intestinal tissue (or of mucosal tissue) separating two solutions *in vitro*.
6	Cross-sectional rings of whole tissue; suspensions of villi isolated from small intestine and incubated *in vitro*.
7	Homogenates of mucosal tissue scraped from the intestine.

defined as digestion, absorption and translocation. Excellent reviews of these achievements are available, (*31-36*); the ground will not be covered again here. Attention will be concentrated on examples of investigations by use of preparations 1-3, since these appear to have advantages not possessed by preparations 5-7 listed in Table 1. Preparation 4 is dealt with elsewhere in this volume (*37*).

Recent Studies on Metabolism in Intestine

Ligature-Isolated Loops of Intestine *in Vivo*; Vascularly and Luminally Perfused Intestine *in Vitro*

Exteriorization of a loop of intestine which is drained by a convenient venous arcade, cannulation of the venous drainage and isolation of the selected length of intestine by two ligatures provides an experimental system which approximates closely to the intestine *in vivo*. Humoral and neural control of intestinal function remain intact, subject only to the effect of the anaesthetic on intestinal metabolism and its regulation. Selected substrates are introduced into the closed loop and homologous blood is introduced by a saphenous vein to replace the blood collected from the venous cannulation of the isolated loop. Provided chemical (as well as any radiochemical) analysis of the collected blood is performed, it is possible to make sound deductions about the rates of translocation of chosen solutes from the lumen across the intestine into the vascular drainage, and about the concomitant metabolic fate of those substrates during their translocation. A further preparation, the vascularly and luminally perfused intestine *in vitro*, has been developed as a highly refined technique in recent years by Hülsmann and colleagues (*38-40*), by Parsons and colleagues (*41-45*), by Windmueller, Spaeth, and Ganote (*46*), and by Windmueller and Spaeth (*47-52*). The perfused intestine *in vitro* suffers from the possible disadvantage that it is not under normal neural and humoral control. It has the advantage over the isolated loop method that it is free of the effects of continuing administration of anesthetics (the surgical procedures required to set up the perfused system last no more than 35 to 45 min, during which time the animal can be ventilated under anesthesia). Furthermore, prolonged perfusion through the vasculature is possible under controlled conditions; a simultaneous or alternative perfusion through the intestinal lumen can be established. The vascular fluid [whole blood (*46*), a suspension of washed erythrocytes in physiological bicarbonate saline (*42*) or an emulsion of fluorocarbon FC75 in physiological bicarbonate saline (*39*)] is saturated with oxygen in an artificial lung and passed once through the intestine or recirculated via the "lung." The luminal perfusing medium is likewise passed once or recirculated. In sum, an extremely versatile system for experimentation is available; absorption of substrates from either or both circuits can be measured, the appearance of products in each circuit can be followed, and the tissue is available for analysis at the end of the experiment. It should be noted that Windmueller, Spaeth, and Ganote (*46*) infused norepinephrine (noradrenalin) and dexamethasone into the vascular fluid during perfusion of the intestine; these infusions were found to be essential for a number of reasons (*46*) and are always included in perfusions of intestine *in vitro* in Windmueller's laboratory (*47-52*). Hülsmann (*38*) included papaverin and promethazin in the vascular perfusion medium; Lamers and Hülsmann (*39*) pretreated animals with papaverin; these reagents were omitted in later experiments (Hülsmann and Porteous, unpublished investigations). Parsons (*41-45*) does not employ pharmacologically active agents during the perfusions. Both Parsons and Windmueller claim to preserve normal tissue histology in the perfused intestines *in vitro*. Windmueller and Spaeth generally starve rats for 18 hr before establishing the perfusion; fed animals are normally used in the other two laboratories. Windmueller and Spaeth generally perfuse the whole of the small intestine and part of the colon; Hanson and Parsons perfuse the jejunum only. Since Windmueller and Spaeth use whole blood as the vascular perfusion medium, several substrates (including glucose) are always presented to the perfused tissue in their experiments; this is not necessarily true in experiments reported from other

laboratories. These variations in perfusion technique may account for some differences in results mentioned below. The original papers should be consulted for full details of surgical procedures, perfusion conditions and for complete discussion of the authors' observations. Selected results only are reviewed here.

Glucose, Fatty Acid, Ketone Body, and Amino Acid Metabolism in the Perfused Intestine

Hülsmann (*38*) showed that glucose (5.2 *mM*) was rapidly removed from the vascular perfusing medium followed, in descending order of removal, by D-3-hydroxybutyrate (5.4 *mM*), octanoate (2 *mM*), and palmitate (1.0 *mM*). The theoretical maximum yields of ATP from the glucose, octanoate and palmitate consumed were of the same order, the theoretical maximum yield from hydroxybutyrate consumed being about 75% of the possible yield from the other substrates (assuming in each case that the substrate consumed was oxidatively catabolised to completion). About 70% of the glucose metabolized was accounted for by lactate. Concomitant consumption of glucose and oleate or octanoate made little difference to the rate of consumption of glucose but lactate then accounted for 96% of the glucose metabolized so that, in this respect, intestine behaved like other tissues. The yield of lactate from glucose as the sole added substrate was remarkably high despite the inclusion of washed erythrocytes (hematocrit = 20%) in the vascular perfusion medium. In later experiments, Lamers and Hülsmann (*39*) replaced erythrocytes with a fluorocarbon emulsion and varied the perfusion technique in several other ways. Conversion of glucose to lactate was diminished to 40-50% under oxygen-saturated conditions and increased to 90% under anaerobic conditions, or upon addition of dinitrophenol under aerobic conditions. Lamers and Hülsmann concluded that the Pasteur effect occurred in intestine. Later observations (Hülsmann and Porteous, unpublished work) added two further pieces of evidence in support of this view. We showed that increasing the oxygen supply to the vascular circuit of perfused rat intestine produced a prompt increase in O_2 consumption and a decrease in lactate production from glucose absorbed from the vascular fluid; the effect was immediately reversed when the oxygen supply was diminished (but still substantial and in the physiological range) and this cycle of events could be repeated during the perfusion. Also, addition of sodium azide (2 *mM*) as a mitochondrial uncoupling agent (*53*) had no detectable effect on the O_2 consumption but produced an immediate marked rise in lactate production, presumably as a response to diminished ATP production from uncoupled mitochondria. Replacement of the azide-containing medium with normal perfusion medium promptly decreased the lactate production to the original low rates. These various pieces of evidence do not provide rigorous proof of the operation of the Pasteur effect; a demonstration of an increased glucose consumption, decreased oxygen consumption, and a simultaneous increase in the conversion of glucose to lactate is required.

In separate work on the glycolytic pathway, Lamers and Hülsmann (*40*) used the rat intestine *in vivo* to show a marked tissue accumulation of fructose-1-phosphate after fructose but not after glucose loading of the lumen. The ATP, ADP, and AMP contents of the tissue remained almost constant whether the lumen was empty, loaded with glucose or loaded with fructose; but under the last circumstances alone, the creatine phosphate content of the tissue was depleted by nearly 45% and the orthophosphate content was diminished by 30%. The authors concluded that in intestinal epithelium, utilization of creatine phosphate compensates for the loss of ATP which would otherwise result when fructose was phosphorylated; and that intestine differs from liver in this respect.

The experiments of Windmueller and Spaeth (*47*) on plasma lipoprotein synthesis will not be discussed, since lipid metabolism in the intestine will be dealt with elsewhere (*54*). In a series of elegant experiments, Windmueller and Spaeth (*48-52*) examined the metabolic fate of glutamate, glutamine, aspartate, asparagine, arginine, and ketone bodies in the perfused rat intestine. The original papers should be consulted for full details of the observations and conclusions. Figures 1-3 attempt to give a graphic summary of some of the more important observations. Thus glucose perfused through the intestinal lumen was apparently translocated largely unchanged into the vascular circulation with about 3% metabolized (Fig. 1); this point will be taken up again later. Of the aspartate which was absorbed from the luminal perfusion medium, only 1% escaped metabolism, the remainder being converted to CO_2, alanine, lactate, and glucose in the proportions shown (other products were also formed in lesser yields). In similar experiments (Fig. 2), Windmueller and Spaeth (*49*) showed that about one third of the glutamine absorbed from the lumen passed unchanged into the vascular circuit, the remainder being metabolized en route through the tissue to the products shown in the proportions indicated. Plasma glutamine was the only amino acid to be withdrawn

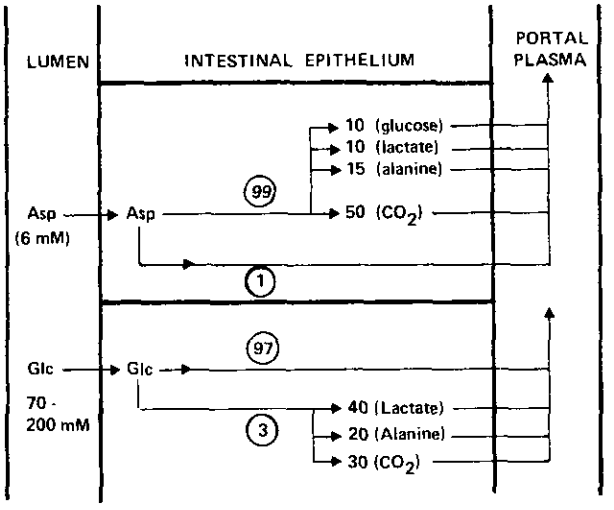

FIGURE 1. Summary of observations by Windmueller and Spaeth (*47, 50*) on the metabolic fate of aspartate and glucose when perfused through the lumen of rat intestine *in vitro;* the vascular bed of the intestine was perfused simultaneously with oxygenated blood. All analytical values have been rounded off for present purposes. Values shown in circles indicate the proportions of absorbed aspartate metabolized and translocated unchanged. Other values shown are the percentage distribution of the metabolised aspartate among the products shown. Results for glucose translocation and metabolism are displayed in the same way; these results are taken in part from the published literature (*47*) and in part from a personal communication from Dr. Windmueller.

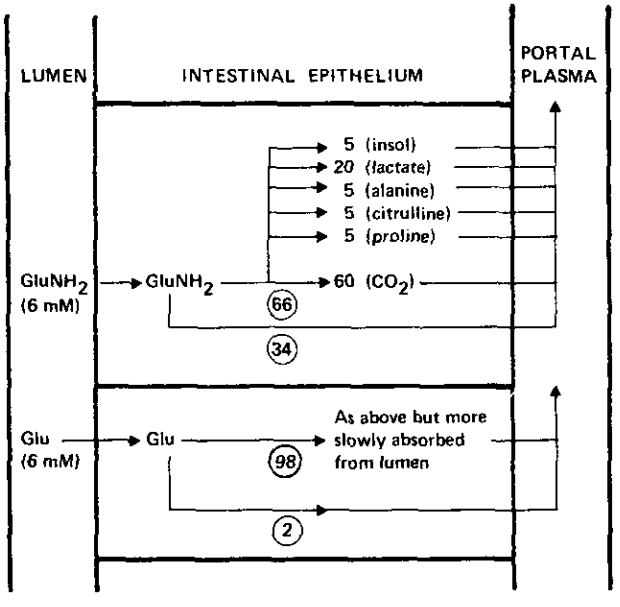

FIGURE 2. Results obtained by Windmueller and Spaeth (*49*) upon perfusing the lumen of ligature-isolated loops of rat jejunum *in vivo* with glutamine or glutamate. The presentation of selected results is analogous to that in Fig. 1.

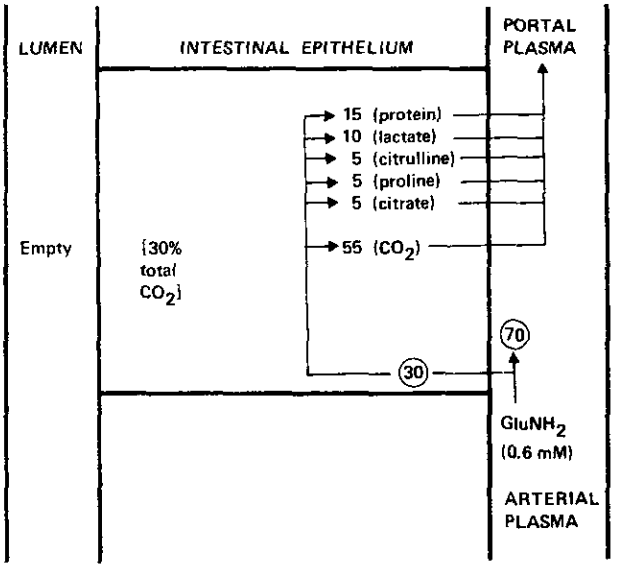

FIGURE 3. Metabolism of plasma glutamine by the vascularly perfused rat intestine. Results taken from Windmueller and Spaeth (*48, 52*) and displayed in the same way as results shown in Figs. 1 and 2.

(Fig. 3) when whole rat blood was perfused through the vascular bed of the rat intestine (*48, 52*); 22-30% was withdrawn upon each pass through the vascular bed so it seemed that rat intestine *in vivo* could play an important role in whole-body metabolism of glutamine. Glutamine withdrawn from the blood perfusing the vascular bed of the intestine was metabolised in the proportions shown to CO_2, citrate, proline, citrulline, lactate, and protein; the CO_2 formed accounted for 30% of the total CO_2 production by the intestine during the perfusion, suggesting (*48, 52*) that glutamine was a major respiratory fuel for the intestine. Further experiments (*52*) revealed the remarkable fact that, at least in intestines taken from rats starved for 18 hr beforehand, 22-30% of arterial plasma glutamine (0.6 *mM*) was withdrawn at each pass and contributed 30% of the total CO_2 produced; of the plasma ketone bodies (0.4 *mM*), 15% was withdrawn by the intestine at each pass and contributed 50% of the total CO_2; only 1% of the plasma fatty acid (0.7 *mM*) was withdrawn and contributed only 3% of the total CO_2; perhaps unexpectedly, only 2% of plasma glucose was withdrawn, despite its presence at much higher concentration than any of the other substrates in the perfusing blood (Table 2), and glucose metabolism contributed only 7% of the total CO_2 produced by the intestine.

Equally elegant and telling results appeared at about the same time from Parsons' laboratory

Table 2. Contribution of plasma substrates to the total CO_2 produced by vascularly perfused rat intestine.[a]

Arterial plasma substrate	Arterial plasma concentration, *mM*	Proportion of plasma flux metabolized, %	Distribution of metabolized substrate among portal plasma products, %		Contribution to total CO_2 produced by intestine *in vivo*, %
Glutamine	0.6	30	CO_2	55	30
			Protein	15	
			Lactate	10	
			Citrulline	5	
			Proline	5	
			Citrate	5	
Ketone bodies	0.4	15	CO_2	65	50
			Lipids	5	
			Amino acids	10	
			Glucose	5	
Nonessential fatty acids	0.7	1	CO_2	25	3
			Tissue lipids	60	
			Acid insolubles	15	
Glucose	7.0	2	CO_2	11	7
			Lactate	55	
			Alanine	16	

[a]Results abstracted from the observations of Windmueller and Spaeth (*52*). The original paper should be consulted for full details of the results and the authors' interpretations.

(*41-45*). Some of these experiments (*41*) have been reviewed elsewhere (*55*) and will not be discussed again here except to recall two key points: (1) a clear demonstration was given of decreased glucose consumption from the vascular circuit, with a concomitant decrease in the proportion of glucose converted to lactate, as the oxygen supply to the intestine was increased; (2) these results could only be achieved when the increased oxygen supply was to the vascular bed of the intestine. These two points provide convincing evidence of the superiority of the perfused intestine over preparations such as the everted sac (Table 1). Taken with the results already reviewed and those to follow, there is no doubt that the perfused intestine *in vivo* and *in vitro* (Table 1) is yielding results of outstanding value.

In later experiments (*43*), Hanson and Parsons examined the effect of placing glucose (or glutamine) alternatively in the vascular and luminal perfusion medium. A few of the results are collated in Table 3 and show that the ratio alanine produced/glutamine utilized alters considerably as the incubation condi-

Table 3. Some results illustrating the effect on alanine and lactate production of changing the initial location or concentration of the substrates (glucose and glutamine) in the vascularly and luminally perfused rat jejunum *in vitro*.[a]

Substrate	Vascular fluid, *mM*	Luminal fluid, *mM*	Glutamine utilized, μmole/g-hr	Glucose utilized, μmole/g-hr	Alanine produced / glutamine utilized, %	Lactate produced / glucose utilized, %
Glucose	7.5	0	75	171	41	38
Glutamine	1.5	0				
Glucose	7.5	0	76	150	67	51
Glutamine	0	4.5				
Glucose	7.5	0	157	162	46	34
Glutamine	1.5	4.5				
Glucose	0	7.5	—	159	—	52
Glucose	7.5	0	—	193	—	33

[a]The results have been selected from several tables in the original publication of Hanson and Parsons (*43*) which should be consulted for further details of these and a wide range of other findings, the statistical analysis of results and the authors' interpretations.

Table 4. Selected results from work by Hanson and Parsons in which glucose was perfused (together with any additions noted) through the vascular bed of rat jejunum *in vitro*.[a]

Glucose (7.5 *mM*) plus:	Glucose utilized, μmole/ g-hr	Lactate produced μmole/ g-hr	Glucose —→ lactate, %
(a) Nothing	170	113	33
(b) β-hydroxybutyrate (2 *mM*) + acetoacetate (1 *mM*)	156	167	52
(c) Na oleate (1 *mM*)	165	114	35
(d) Nothing — diabetic rats	120	118	51

[a]Donor rats were fed. Further details are found in the original publication (*45*).

tions were varied. The ratio lactate produced/ glucose utilized likewise varies with conditions of incubation. Hanson and Parsons drew attention to the increased proportion of glucose converted to lactate when glucose was in the luminal rather than in the vascular circuit. Of the glucose (7.5 *mM*) absorbed from the intestinal lumen, 44% passed unchanged into the vascular circulation; of the 56% which was metabolized, 52% was accounted for as lactate (Table 3). These results (*43*) stand in marked contrast to those obtained by Windmueller and Spaeth under somewhat different conditions (Fig. 1). The dominant role of ketone bodies as a respiratory fuel of the perfused intestine, at least when the rat had been starved for 18 hr beforehand, will be recalled (Table 2). Hanson and Parsons (*45*) examined the effect of oleate and ketone bodies upon the conversion of glucose to lactate by vascularly perfused intestines taken from fed and starved rats; selected results are shown in Tables 4 and 5. Adding oleate to the medium perfusing the vascular bed of intestines from fed rats had no effect on the proportion of glucose converted to lactate; but in intestines from diabetic rats or in normal intestine perfused additionally with ketone bodies, there was a marked increase in the proportion of glucose converted to lactate. In the intestine of starved rats there was an increased conversion of glucose to lactate in the unsupplemented medium; addition of oleate now produced a significant increase in the proportion of glucose metabolized to lactate, and addition of ketone bodies resulted in a complete conversion of glucose to lactate.

It will be recalled (Fig. 1) that Windmueller and Spaeth found only a small conversion of luminal glucose to lactate during translocation of the glucose through intestine. In all other experiments reviewed here (Tables 2-5) lactate was a prominent product of glucose metabolism. It remains to be seen whether any of the variation in perfusion conditions summarised earlier in this article were responsible for part of this discrepancy; Windmueller and Spaeth (*52*) report that a major reason for the apparent lack of formation of net amounts of lactate in their earlier experiments (*47*) was that the tissue was simultaneously consuming lactate which happened to be present at relatively high concentrations in the vascular circuit in those experiments.

In sum, work with the perfused intestine over less than a decade has already added significantly to our understanding of the capacity of the tissue to withdraw and metabolise substrates from the vascular bed and/or from the intestinal lumen (Figs. 1-3; Tables 2-5). The tissue respires effectively only when supplied with oxygen from the vascular bed (*42*). It responds promptly and reversibly to any fluctuations in this supply of oxygen. A given substrate supplied alternatively from the lumen or the vascular bed and absorbed by the tissue is generally metabolised by qualitatively similar routes but its quantitative fate may be different. The tissue responds remarkably to short-term starvation and retains its starvation-adapted metabolic capacities *in vitro*. Glutamine, ketone bodies, fatty acids, and glucose appear to be potential major respiratory fuels, but there remain discrepancies in the relative importance ascribed by different authors to these substrates of respiration. There have been relatively few direct measurements of O_2 consumption reported in conjunction with measurements of the catabolism of these substrates in perfused preparations of intestine.

Metabolism of Glucose, Amino Acids, and Ketone Bodies by Suspensions of Isolated Intestinal Brush Border Cells

Given the characteristics of the intestine described in the Introduction to this article, and the efficacy of the perfused intestine illustrated in the preceding

Table 5. Selected results from work by Hanson and Parsons in which glucose was perfused (together with any other additions noted) through the vascular bed of rat jejunum *in vitro*.[a]

Glucose (7.5 *mM*) plus:	Glucose utilized, μmole/ g-hr	Lactate produced, μmole/ g-hr	Glucose —→ lactate, %
(a) Nothing	62	59	46
(b) β-Hydroxybutyrate (2 *mM*) + acetoacetate (1 *mM*)	57	118	113
(c) Na oleate (1 *mM*)	90	110	63

[a]Donor rats were starved 48 hr before the experiment. Further details are given in the original publication (45).

section, it may be asked whether work with isolated cells has any value. It is clear that suspensions of brush border cells cannot reveal the quantitative differences in metabolic fate of a given substrate which have been shown by perfusing substrates alternatively through the lumen and the vascular bed of the intestine. Suspensions of brush border cells may nevertheless be useful for metabolic and other studies for the following reasons: they are easily and rapidly prepared in good yield from any selected part of the intestine; subpopulations of the differentiating brush border cells can be isolated from the intestinal villi and subpopulations of proliferating and differentiating crypt cells can also be prepared if required (*56*); aliquots of the cell suspension can readily be distributed to permit simultaneous investigation of the metabolism of several different substrates; simultaneous continuous measurements of respiration are readily performed; comparative work with several different species of intestine is easily achieved as are experiments comparing cells from different regions of the same intestine; experimental results are clearly ascribable to the epithelium and not to any other intestinal tissue component.

Provided, then, that the limitations of the use of cell suspensions are borne in mind, the technique should prove useful. Experience shows that this is the case. In this author's view, the perfusion technique and the isolated cell method are complementary; they are not in competition.

An absolute prerequisite of the use of cell suspensions is that their origin in the tissue should be identified, their purity assessed, their morphological and biochemical integrity quantified. The required assessments have been published (*57*). Particular attention is drawn to the existence of tight junctions and desmosomes between adjacent cells (see the Introduction to this article); the primary isolates from intestine then necessarily consist of sheets of substantial numbers of cells arranged in pallisade fashion. In the author's experience, it has so far proved impossible to prepare dispersed suspensions of intact individual cells; such individual cells as do appear under microscopic examination of isolated material are almost invariably damaged cells. Occasionally, a cluster of two or three intact cells can be found near the edge of a sheet of cells and such cells reveal clearly their characteristic brush border more readily than do the sheets of cells. Crypt cells, in contrast, are much smaller, lack a brush border and are more readily isolated as suspensions of individual cells.

We have shown (*57*) that addition of glucose (10 *mM*) stimulated endogenous respiration of rat jejunal brush border cells by 70%; addition of L-glutamine (5 *mM*) stimulated respiration by 35%. Lactate was a major product and alanine a minor product of glucose metabolism; cells maintained a so-called "energy charge" of 0.73 during respiration on glucose, the mass-action ratio $[ATP]/[ADP][P_i]$ was 365 under the same circumstances, and the adenine nucleotide content of the cells varied in a predictable way when cells were subjected to several treatments. The cells accumulated and discharged non-metabolized substrates in a predictable way. These observations (*57*) provide some assurance that the cells as isolated were intact and suitable tools for investigation of metabolism by intestinal epithelium.

Watford, Lund, and Krebs (*58*) studied the metabolism of glucose, glutamate, glutamine, aspartate, lactate, pyruvate and acetoacetate added to suspensions of respiring rat jejunal brush border cells. Of these substrates, glucose (10 *mM*) stimulated endogenous respiration by 100%, glutamine (5 *mM*) by 60%, glutamate (5 *mM*) by 60%, acetoacetate (5 *mM*) by 30%, pyruvate (10 *mM*) by 22%, and pyruvate (1 *mM*) + lactate (10 *mM*) by 34%. Addition of oleate (1 *mM*), butyrate (5 *mM*), propionate (2 *mM*), or acetate (5 *mM*) did not stimulate respiration above the rate observed on endogenous substrates. Much of the glutamine consumed appeared as glutamate, alanine, aspartate and NH_3. Glutamate was metabolized much more slowly than was glutamine. About half of the glutamate consumed appeared as equimolar amounts of alanine and NH_3 plus a smaller amount of aspartate. Of the glucose metabolized by the respiring cells, 70% appeared as lactate.

These results alone encourage the belief that isolated brush border cells provide a convenient means of surveying the metabolic capacity of intestinal epithelium. The results obtained are consistent with, but do not duplicate, those obtained with the perfused intestine. Further extensive studies from the same laboratory using suspensions of chick brush border cells provide substantial further encouragement (Porteous, paper in preparation). Two results bearing directly on those obtained with perfused intestines in other laboratories are mentioned here.

The proportion of glucose metabolized to lactate by respiring perfused intestine can vary (Fig. 1, Tables 4 and 5) from 33 to 100% depending upon the initial location of the glucose, the nutritional status of the animal donor, the presence or absence of other substrates and the precise conditions of perfusion. Results with respiring chick intestinal brush border cells suggest that the initial concentration of the glucose may also affect the proportion of glucose con-

Table 6. Effect of initial concentration of glucose on the rate of respiration and on the proportion of glucose converted to lactate by respiring chick jejunal brush border cells.

Substrate consumed or product[a] formed (net)	Metabolic rates at various initial glucose concns, μmole/min/g dry wt	
	5.0 *mM* glucose	10.0 *mM* glucose
Oxygen (endog: −14.0)	− 4.7	− 1.4
Glucose	−13.1	−10.3
Pyruvate	+ 1.2	+ 1.5
Lactate	+16.6	+19.2
Lact/Pyr	13.8	12.8
Glucose → Lactate (%)	63	93

[a]Consumption of substrate indicated by negative metabolic rate (right); formation of product by positive rate.

verted to lactate (Table 6). These chick cells exhibit a respiration rate on endogenous substrates (14 μmole O_2/min/g dry weight) which is 40% greater than the corresponding rate in rat cells. Upon addition of glucose (5 *mM*) the gross rate of respiration by chick cells was 18.7 μmole/min/g dry weight cells, and 63% of the glucose consumed appeared as lactate. The same cells, when incubated with 10 *mM* glucose, still respired vigorously (gross rate 15.4 instead of 18.7 μmole/min/g dry weight) but at a significantly slower rate than that observed with the lower glucose concentration; furthermore, glucose was consumed more slowly. The ratio lactate produced/pyruvate produced was, if anything, slightly lower in the presence of the higher concentration of glucose; at least there was no sign that the cytosolic ratio NADH/NAD^+ was increased under these circumstances. Yet, in the presence of 10 *mM* glucose these respiring cells unexpectedly but consistently converted a greater proportion of the metabolised glucose to lactate than was apparent when the initial glucose concentration was 5 *mM*. Postulates have been made elsewhere about mechanisms regulating the proportions of glucose catabolized to CO_2 and lactate in the intestine (*59*). Detailed results of the kind illustrated in Table 6 are perhaps more readily obtained with cell suspensions than with a perfused tissue. Other evidence for changes in metabolic patterns as the initial concentrations of substrates were varied will be presented elsewhere.

Windmueller and Spaeth (*48*) suggested that rat intestine might synthesize glutamine as well as consume and degrade it. Direct evidence that this synthesis occurs in chick jejunal brush border cells has now been obtained; some of that evidence is summarized in Figure 4. In essence, when glutamate or glutamine is incubated separately at low concentrations with intestinal brush border cells, the overall reactions favor glutamine synthesis. When the initial concentrations of glutamine and glutamate are raised, the reactions favor overall conversion of glutamine to glutamate. These observations do not provide a full explanation for the observed balance in favor of net consumption of glutamine by the perfused intestine (*43, 45, 48, 49, 51, 52*); further evidence and discussion will be given elsewhere.

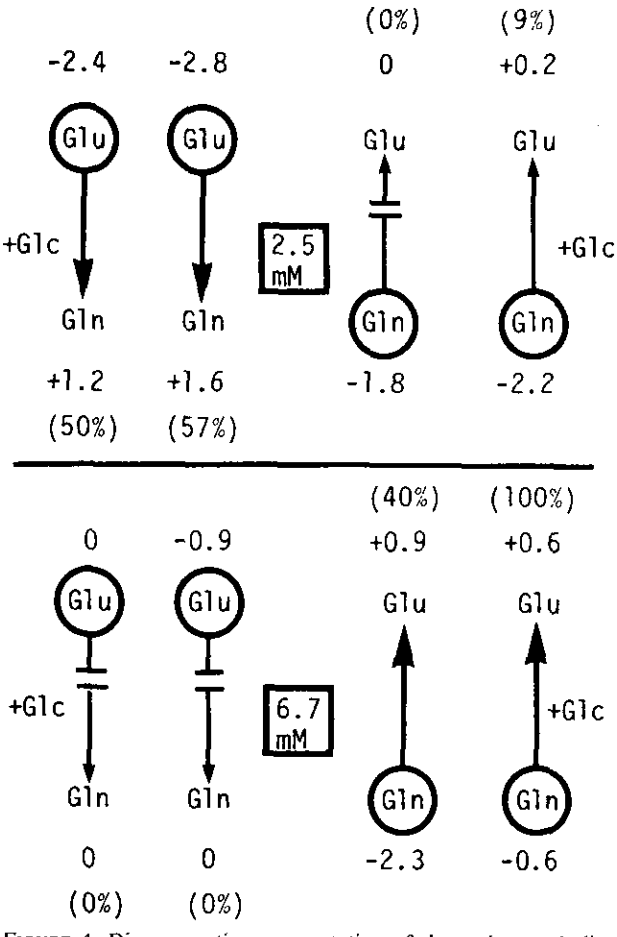

FIGURE 4. Diagrammatic representation of the main metabolic fates of glutamate (Glu) and glutamine (Gln) when incubated alone or in the presence of glucose (+Glc) with suspensions of respiring chick jejunal brush border cells. Substrates are shown within circles; the values shown against the substrates are net rates of consumption (μmol/min g dry weight cells). Values shown against the products formed are the net rates of production; the percentage yield of product is also indicated. In one set of experiments, the initial concentration of substrate was 2.5 *mM* as indicated; in the other, it was 6.7 *mM* as indicated. Results obtained by the author in Professor Krebs' laboratory.

Conclusions

Three relatively new and potent experimental systems have been described for the investigation of scalar and vectorial metabolism in intestine (Table 1). The results reviewed give new insight into the metabolic capacities of the intestine and suggest that, in respect of some plasma substrates, intestine must play a major role in whole body metabolism over and above its established roles in the terminal stages of digestion and in translocation of nutriments from the lumen to the portal circulation. Each of the three preparations of intestine described should provide excellent tools for screening pharmaceuticals, herbicides, pesticides, preservatives and other compounds known to present hazards to the human population, farmstock and wildlife. There is little doubt that liver has been and will continue to be a test bed for such studies. But there is no reason to believe that liver, or any other organ, enjoys a special or sole prerogative in dealing with all xenobiotics. In many circumstances, intestine is one of the first tissues to suffer insult from ingested foreign compounds.

The author's work in Aberdeen has been generously supported by the Medical Research Council, London and by the Pooled Medical Endowments Fund of the University of Aberdeen.

I wish also to thank the Ciba-Geigy Fellowship Trust for the award of a Senior Research Fellowship, and the Medical Research Council for funds to defray laboratory expenses during sabbatical leave from this University.

It is a particular pleasure to thank Professor Willem Hülsmann and his colleagues for their hospitality and willing help during a short stay in the Rotterdam Laboratory. I am also much indebted to Professor Sir Hans Krebs and all other members of the Metabolic Research Laboratory, Radcliffe Infirmary, Oxford for generous hospitality during a memorable spell in that laboratory.

REFERENCES

1. Greene, E. C. The Anatomy of the Rat. (Amer. Phil. Soc. Trans. Vol. 27), Hafner, London and New York, 1963.
2. Farquhar, M. G., and Palade, G. E. Junctional complexes in various epithelia. J. Cell Biol. 17: 375 (1963).
3. Staehelin, L. A. and Hull, B. E. Junctions between living cells. Sci. American 238: 141 (1978).
4. Winne, D. The vasculature of the jejunal villus. In: Intestinal Permeation (Workshop Conference Hoechst, Vol. 4), M. Kramer and F. Lauterbach, Eds., Excerpta Medica, Amsterdam and Oxford, 1977.
5. Greenway, C. V., and Lawson, A. E. The effects of adrenaline and noradrenaline on venous return and regional blood flows in the anaesthetized cat with special reference to intestinal blood flow. J. Physiol. 186: 579 (1966).
6. Jodal, M., and Lundgren, O. Plasma skimming in the intestinal tract. Acta Physiol. Scand. 80: 50 (1970).
7. Levin, R. J. The effects of hormones on the absorptive, metabolic and digestive functions of the small intestine. J. Endocrinol. 45: 315 (1969).
8. Trier, J. S. and Rubin, C. E. Electron microscopy of the small intestine: a review. Gastroenterol. 49: 574 (1965).
9. Toner, P. G. Cytology of intestinal epithelial cells. Internat. Rev. Cytol. 24: 233 (1968).
10. Toner, P. G., Carr, K. E., and Wyburn, G. M. The Digestive System — An Ultrastructural Atlas and Review. Butterworths, London, 1971.
11. Sassier, P., and Bergeron, M. Cellular changes in the small intestine epithelium in the course of cell proliferation and maturation. Subcellular Biochem. 5: 129 (1978).
12. Specht, W. Morphology of the intestinal wall: its mucous membrane under normal and experimental conditions. In: Intestinal Permeation (Workshop Conference Hoechst, Vol. 4), M. Kramer and F. Lauterbach, Eds., Excerpta Medica, Amsterdam and Oxford, 1977.
13. Potten, C. Epithelial proliferative subpopulations. In: Stem Cells and Tissue Homeostasis, B. I. Lord, C. S. Potten, and R. J. Cole, Eds., Cambridge Univ. Press, Cambridge, England, 1978.
14. Wright, N. A. The cell population kinetics of repopulating cells in the intestine. In: Stem Cells and Tissue Homeostasis, B. I. Lord., C. S. Potten, and R. J. Cole, Eds., Cambridge Univ. Press, Cambridge, England, 1978.
15. Cheng, H., and Leblond, C. P. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cells. Am. J. Anat. 141: 461 (1974).
16. van Dongen, J. M., Visser, W. J., Daems, W. T., and Galjaard, H. The relation between cell proliferation, differentiation and ultrastructural development in rat intestinal epithelium. Cell Tissue Res. 174: 183 (1976).
17. Burholt, D. R., Schultze, B., and Maurer, W. Autoradiographic confirmation of the mitotic division of every mouse jejunal crypt cell labelled with ^{3}H-thymidine. Evidence against the existence of cell synthesizing metabolic DNA. Cell Tissue Res. 6: 229 (1973).
18. Grey, R. S., and LeCount, T. S. Distribution of leucyl naphthylamidase and alkaline phosphatase on the villi of chick duodenum. J. Histochem. Cytochem. 18: 416 (1970).
19. Nordström, C., Dahlqvist, A., and Josefsson, L. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. J. Histochem. Cytochem. 15: 713 (1968).
20. Imondi, A. R., Balis, M. E., and Lipkin, M. Changes in enzyme levels accompanying differentiation of intestinal epithelial cells. Exptl. Cell Res. 58: 323 (1969).
21. Petersen, A. E. Differentiation associated changes in thymidylate synthetase and thymidine kinase activities in intestinal cells. Cell Differentiation 5: 37 (1976).
22. Kim, Y. S., Perdomo, J., Ochoa, P., and Isaacs, R. A. Regional and cellular localization of glycosyltransferases in rat small intestine. Changes in enzymes with differentiation of intestinal epithelial cells. Biochim. Biophys. Acta 391: 39 (1975).
23. Hülsmann, W. C., and Kurpershoek-Davidov, R. Topographic distribution of enzymes involved in glycerolipid synthesis in rat small intestinal epithelium. Biochim. Biophys. Acta 450: 288 (1976).
24. Simon, P. M., Kedinger, M., Raul, F., Grenier, J. F., and Haffen, K. Developmental pattern of rat intestinal brush-border enzymic proteins along the villus-crypt axis. Biochem. J. 178: 407 (1975).
25. Rosenweig, N. S. Diet and intestinal enzyme adaptation; implications for gastrointestinal disorders. Am. J. Clin. Nutrit. 28: 648 (1975).
26. Desai, I. D. Regulation of lysosomal enzymes. II. Reversible adaptation of intestinal hydrolases during starvation and realimentation. Can. J. Biochem. 49: 170 (1971).
27. Saito, M., Murakami, E., Nishida, T., Fujisawa, Y., and Suda, M. Circadian rhythms in digestive enzymes in the small intestine of rats. J. Biochem. 78: 475 (1975).

28. Schiller, C. M., and Lucier, G. W. The differential response of isolated intestinal crypt and tip cells to the inductive actions of 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin. Chem. Biol. Interact. 22: 199 (1978).
29. Stohs, S. J., Grafstrom, R. C., Burke, M. D., and Orrenius, S. Benzo (α) pyrene metabolism by isolated rat intestinal epithelial cells. Arch. Biochem. Biophys. 179: 71 (1977).
30. Lee, D. H. K., Ed. Handbook of Physiology. Xenobiotics: Section 9, Reactions to environmental agents, Amer. Physiol. Soc., Washington, D.C. 1977, pp. 349-389.
31. Code, C. F. and Heidel, W., Eds. Handbook of Physiology: Section 6, Alimentary Canal, Vol. III, Intestinal Absorption, Amer. Physiol. Soc., Washington, D.C. 1968.
32. Burland, W. L., and Samuel, P. D., Eds. Transport Across the Intestine (A Glaxo Symposium), Churchill Livingstone, Edinburgh and London, 1972.
33. Matthews, D. M. Absorption of water-soluble vitamins. In: Biomembranes, Vol. 4B, Intestinal Absorption, D. H. Smyth, Ed., Plenum Press, London and New York, 1974.
34. Matthews, D. M. Intestinal absorption of peptides. Physiol. Rev. 55: 537 (1975).
35. Matthews, D. M., and Payne, J. W., Eds. Peptide Transport in Protein Nutrition. North-Holland Research Monographs; Frontiers of Biology, Vol. 37, North Holland, Amsterdam, 1975.
36. Kramer, M. and Lauterbach, F., eds. Intestinal Permeation (Workshop Conference Hoechst, Vol. 4), Section II, IV, V, Excerpta Medica, Amsterdam and Oxford, 1977.
37. Crane, R. K. Intestinal structure and function related to toxicology. Environ. Health Perspect. 33: 3 (1979).
38. Hülsmann, W. C. Preferential oxidation of fatty acids by rat small intestine. FEBS Letters 17: 35 (1971).
39. Lamers, J. M. J., and Hülsmann, W. C. Pasteur effect in the *in vitro* vascularly perfused rat small intestine. Biochim. Biophys. Acta 275: 491 (1972).
40. Lamers, J. M. J., and Hülsmann, W. C. The effect of fructose on the stores of energy-rich phosphate in rat jejunum *in vivo*. Biochim. Biophys. Acta 313: 1 (1973).
41. Parsons, D. S., and Powis, G. Some properties of a preparation of rat colon perfused *in vitro* through the vascular bed. J. Physiol. 217: 641 (1971).
42. Hanson, P. J., and Parsons, D. S. The utilization of glucose and production of lactate by *in vitro* preparations of rat small intestine: effects of vascular perfusion. J. Physiol. 255: 775 (1976).
43. Hanson, P. J., and Parsons, D. S. Metabolism and transport of glutamine and glucose in vascularly perfused rat small intestine. Biochem. J. 166: 509 (1977).
44. Boyd, C. A. R., and Parsons, D. S. Effects of vascular perfusion on the accumulation, distribution and transfer of 3-O-methyl-D-glucose within and across the small intestine. J. Physiol. 274: 17 (1978).
45. Hanson, P. J., and Parsons, D. S. Factors affecting the utilization of ketone bodies and other substrates by rat jejunum: effects of fasting and of diabetes. J. Physiol. 278: 55 (1978).
46. Windmueller, H. G., Spaeth, A. E., and Ganote, C. E. Vascular perfusion of isolated rat gut: norepinephrine and glucocorticoid requirement. Am. J. Physiol. 218: 197 (1970).
47. Windmueller, H. G., and Spaeth, A. E. Fat transport and lymph and plasma lipoprotein biosynthesis by isolated intestine. J. Lipid. Res. 13: 92 (1972).
48. Windmueller, H. G., and Spaeth, A. E. Uptake and metabolism of plasma glutamine by the small intestine. J. Biol. Chem. 249: 5070 (1974).
49. Windmueller, H. G., and Spaeth, A. E. Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from the blood. Arch. Biochem. Biophys. 171: 662 (1975).
50. Windmueller, H. G. and Spaeth, A. E. Metabolism of absorbed aspartate, asparagine and arginine by rat small intestine *in vivo*. Arch. Biochem. Biophys. 175: 670 (1976).
51. Windmueller, H. G., and Spaeth, A. E. Vascular perfusion of rat small intestine: metabolic studies with isolated and in situ preparations. Fed. Proc. Fed. Amer. Soc. Exp. Biol. 36: 177 (1977).
52. Windmueller, H. G., and Spaeth, A. E. Identification of ketone bodies and glutamine as the major respiratory fuels *in vivo* for postabsorptive rat small intestine. J. Biol. Chem. 253: 69 (1978).
53. Mitchell, P. Vectorial chemistry and the molecular mechanics of chemiosmotic coupling: power transmission by proticity. Biochem. Soc. Trans. 4: 399 (1976).
54. Kuksis, A., Shaikh, N. A., and Hoffman, A. G. D. Lipid absorption and metabolism, Environ. Health Perspect. 33:45 (1979).
55. Porteous, J. W. Glucose as a fuel for small intestine. Biochem. Soc. Trans. 6: 534 (1978).
56. Porteous, J. W., Furneaux, H. M., Pearson, C. K., and Lake, C. M. Poly- (adenosine diphosphate ribose) synthetase activity in nuclei of dividing and of non-dividing but differentiating intestinal epithelial cells. Biochem. J. 180: 455 (1979).
57. Towler, C. M., Pugh-Humphrey, G. P., and Porteous, J. W. Characterization of columnar absorptive epithelial cells isolated from rat jejunum. J. Cell Sci. 29, 53 (1978).
58. Watford, M., Lund, P., and Krebs, H. A. Isolation and metabolic characteristics of rat and chicken enterocytes. Biochim. J. 178: 589 (1979).
59. Porteous, J. W. In: Intestinal Permeation (Workshop Conference Hoechst, Vol. 4), M. Kramer and F. Lauterbach, Eds., Excerpta Medica, Amsterdam and Oxford, 1977.